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PRINCIPAL INVESTIGATOR: Jean J. Zhao, Ph.D.

CONTRACTING ORGANIZATION: Dana-Farber Cancer Institute
Boston, MA 02115

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Jean J. Zhao, Ph.D.

Email: jean_zhao@dfci.harvard.edu

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14. ABSTRACT

Our research described here is relevant to the pathogenesis and a potential novel therapy for breast cancers. The PIK3CA is the most commonly mutated oncogene in breast cancer and loss of the tumor suppressor, PTEN, occurs frequently in patients suffering from this disease. The most significant accomplishment during the first year of funding is the generation of the oncogenic PIK3CA transgenic animal model which will allow us to determine the oncogenic role of PIK3CA in tumor initiation, progression, maintenance and metastasis etc. It should also significantly facilitate preclinical testing for the development of PI3K inhibitors for targeted therapy. Our final goal of simultaneous inactivation of PTEN and activation of PIK3CA will not only provide a new perspective on the relationship of the two key oncogene and tumor suppressor, PIK3CA and PTEN, and the signaling pathway under their control in cell regulation and oncogenic transformation, but also a potential novel therapy to all patients plagued with the common tumorigenic mutations.

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Introduction

Our proposed study is to test a novel molecular targeting strategy that challenges the existing paradigm of drug treatment: Instead of inhibiting PI3K activity and reduce the amount of PIP_3 in cancer cells carrying activating PI3K mutants, we will further elevate the PIP_3 levels by inactivating PTEN to push these tumor cells "over the brink". The lipid PIP_3 is a critical second messenger in cell signal transduction. The level of PIP_3 is tightly regulated by the activities of these two opposing enzymes, the lipid kinase, PI3K, and the lipid phosphatase, PTEN. We believe that PI3K activity is tolerated within a relatively narrow window in cells - "too much of PIP_3 is just as lethal as too little". The abnormal elevation of PIP_3 levels has been frequently found in human cancers bearing somatic activating mutations in the *PIK3CA* gene or loss of PTEN function. Interestingly, while both *PIK3CA* and *PTEN* mutations occur so frequently in cancers (Bachman et al., 2004; Samuels et al., 2005), *PIK3CA* mutations and *PTEN* loss are almost mutually exclusive in, at least, breast (Saal et al., 2005), brain (Broderick et al., 2004), and gastric cancers (Byun et al., 2003). Since the two genes act as "on/off" switches on PI3K signaling, the reciprocal nature of *PIK3CA* mutations and *PTEN* inactivation indicate that while either *PIK3CA* activation or *PTEN* loss of function results in an elevation of PIP_3 sufficient for oncogenisis, the *PIK3CA*/PTEN double mutants may elevate PIP_3 to a lethal level. To test this hypothesis, we will inactivate *PTEN* in cells expressing activating mutants of *PIK3CA*. We will also test our idea by simultaneous activation of PI3K and inactivation of *PTEN* in an animal breast tumor model.

Aim 1. To determine the effect of *PTEN* inactivation in human mammary epithelial cells (HMECs) expressing activated alleles of *PIK3CA.*

Aim 2. To determine the effect of simultaneous inactivation of *PTEN* and activation of *PIK3CA* in an animal breast tumor model.

Body

During the first year of funding, we have devoted most of our effort in testing the inactivation of PTEN in HMECs and generating the transgenic mouse carrying the oncogenic *PIK3CA* under a inducible tetO-promoter as outlined in the Statement of Work (SOW). We have made good progresses, especially in making the transgenic animal models for Aim 2, although encountered some difficulties in Aim 1 with *PTEN* inactivation. Details of the studies and results are as follows:

<u>On Aim1: Inactivation of *PTEN* using RNAi in HMECs.</u> To test the effect of transient inactivation of *PTEN* in cells, we have tried introducing *PTEN*-siRNAs oligos into HMECs by transfection. However, these HMECs were extremely difficulty to be transfected. With several leading transfection regents used, the highest efficiency of transfection to HMECs was only near 10%. Such low transfection efficiency affected our further analysis of the correlation of PTEN silencing and cell death and survival. Using retro-viral based shRNA (pSuper-retroviral-shPTEN) mediated knockdown, we have generated three stable HMEC lines transduced with three retroviral shPTENs targeting different regions of *PTEN* mRNA. Western-blot analysis showed that the best reduction of PTEN level was about 60% as compared to that of control

cells. We found the 60% reduction of PTEN level was not sufficient to induce anchorage-independent growth of HMECs in the presence of hTERT, high level of c-my and p53DD. We have then tested a set of five lentiviral shRNAs against *PTEN* and found one of them significantly knockdown PTEN with close to 90% efficiency. We are now constructing a lenti-based inducible H1 vector carrying this working shRNA-PTEN. We have already generated lentiviral construct bearing tetR and made a stable HMEC line expressing tetR which will be introduced with inducible shRNA-PTEN for the functional analysis listed in task 1B of SOW.

On Aim 2: Simultaneous inactivation of PTEN and activation of PIK3CA in a mouse mammary tumor model. We have succeeded in making the transgenic constructs carrying inducible expression of tumor mutant alleles of *PIK3CA, H1047R or E545K,* and luciferase for live imaging of breast tissue: TetO-HA-PIK3CA-IRES-luciferase (Fig. 1). More than 60-fold and 80-fold induction were reached as tested by transfecting 293-tetR cells with the "TetO-HA-PIK3CA-IRES-luciferase" constructs and treated with doxycycline or untreated (Fig. 2). The construct, TetO-HA-H1047R-IRES-luciferase (T-H1047R-luc), was picked for the production of our transgenic line by injecting the DNA into embryos followed by implantation into foster mice. This was done at Harvard Medical School Transgenic Core Facility. Genotyping of tail biopsies showed that five pups were transgenic founders, including 3 males and 2 females. All three male founders were bred and produced progenies carrying the transgene, confirming positive germline transmission. The two female founders have not produced after being in a breeder for three months. The transferring of MMTV-rtTA mouse line (Gunther et al., 2002) from Dr. Chodosh's lab is in process. This MMTV-rtTA line will be crossed with the newly produced T-H1047R-luc transgenic line to generate a bi-tansgenic mouse, T-H1047R-luc:MMTV-rtTA. This model will allow us to determine the oncogenic role of *PIK3CA* in tumor development and, ultimately, examine the simultaneous inactivation of PTEN and activation of PIK3CA in mouse mammary tumorigenesis.

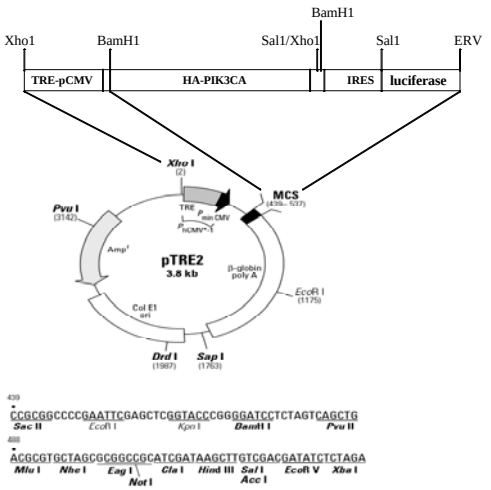

Fig. 1 Construction of the transgenic vector carrying the expression cassette "TetO-HA-PIK3CA-IRES-luciferase".

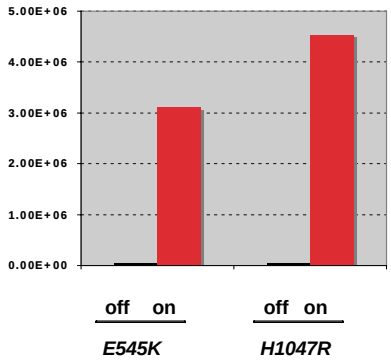

Fig. 2. Luciferase report assay showing the high inductions were reached in the presence of doxycycline.

Both of the PTEN flox and MMTV-Cre mouse lines (Li et al., 2002; Wagner et al., 1997) have been obtained from NCI-Frederick Repository and completed quarantine at Charles River Laboratory animal facility. We have bred the PTEN flox to homozygous PTEN $^{Lox/Lox}$, and started crossing the PTEN flox and MMTV-Cre lines to generate PTEN $^{Lox/Lox}$ /MMTV-Cre as described in SOW.

Key Research Accomplishments

1. Constructed pLenti-rtTA vector.
2. Generated a stable HMEC line expressing tetR.
3. Constructed transgenic vectors pTetO-HA-H1047R-IRES-luciferase and pTetO-HA-PIK3CA-IRES-luciferase.
4. Generated transgenic mouse line carrying TetO-HA-H1047R-IRES-luciferase (T-H1047R-luc).

Reportable Outcomes

The support from the Department of Defense Idea Award has helped me to start my independent research program and provided research opportunity for one postdoctoral fellow, Hailing Cheng, to pursue her career in cancer research. Other outcomes, such as cell lines and animal models, are listed under "key research accomplishments"

Conclusion

The research described here is relevant to the pathogenesis and a potential novel therapy for breast cancers. The *PIK3CA* is the most commonly mutated oncogene in breast cancer and loss of the tumor suppressor, PTEN, occurs frequently in patients suffering from this disease. Our newly generated oncogenic *PIK3CA* transgenic animal model will allow us to determine the oncogenic role of *PIK3CA* in tumor initiation, progression, maintenance and metastasis etc. It should also significantly facilitate preclinical testing for the development of PI3K inhibitors for targeted therapy. Our final goal of simultaneous inactivation of *PTEN* and activation of *PIK3CA* will not only provide a new perspective on the relationship of the two key oncogene and tumor suppressor, *PIK3CA* and *PTEN*, and the signaling pathway under their control in cell regulation and oncogenic transformation, but also a potential novel therapy to all patients plagued with the common tumorigenic mutations.

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Appendices None